Medical Progress

New Developments in the Treatment of Gram-Negative Bacteremia

MARK A. JACOBSON, MD, *Los Angeles, and* LOWELL S. YOUNG, MD, *San Francisco*

Gram-negative bacteremia is an increasingly important nosocomial infectious problem. Endotoxin, endorphins, leukocyte agglutination and deficient opsonization all appear to be major factors in the pathogenesis of Gram-negative septic shock. Outcome has previously correlated best to underlying disease state. With appropriate double antibiotic therapy and hemodynamic support, however, mortality has decreased even for neutropenic patients. Corticosteroids, naloxone, granulocyte transfusions and immunotherapy are experimental adjunctive modes of therapy that offer hope for even better survival in the future.

(Jacobson MA, Young LS: New developments in the treatment of Gram-negative bacteremia [Medical Progress]. West J Med 1986 Feb; 144:185-194)

Gram-negative bacteremia complicating medical and surgical therapy has become one of the major current in-hospital infectious problems. It is a medical emergency requiring intervention aimed at eliminating bacteremia and reversing pathophysiologic consequences that can result in shock and death. Although many of the signs and symptoms of nosocomial Gram-negative bacteremia are similar to those seen in Gram-positive bacteremia and fungemia, it is best to view this serious problem as a distinct clinical entity.

Incidence

Cases of Gram-negative bacteremia due to *Escherichia coli* and *Pseudomonas* were first described at the turn of the century. At that time, these were considered medical curiosities that often occurred in instrumented or debilitated patients. By 1920 fewer than 100 cases had been described. The first major study of sepsis due to Gram-negative bacilli other than *Salmonella* or *Yersinia* was published in 1951.[1] Most of the cases described in that study were associated with instrumentation or prior antibiotic therapy.

Currently, the incidence of Gram-negative bacteremia appears to be increasing. Although few medical centers have kept records for more than a decade, Boston City Hospital has reported that between 1957 and 1972 the incidence of *E coli* bacteremia increased fivefold. The incidence of Gram-negative bacilli bacteremia is currently estimated at 70,000 to 300,000 cases per year, with a mortality rate of 19% to 50%.[2-4]

Source of Infection

In humans, the gastrointestinal tract is a reservoir for Gram-negative bacilli and is probably the source of most serious Gram-negative infections with these organisms. It is the obvious source of biliary sepsis and postsurgical abdominal infections. Fecal contamination of the urethra is the source of most cases of pyelonephritis. Small ulcerations of the gastrointestinal tract are believed to be the source of bacteremia in immunosuppressed patients who lack an obvious source. Fecal carriage of more virulent Gram-negative bacilli such as *Pseudomonas aeruginosa* increases with antibiotic use and neutropenia. It has been shown that the oropharynx of debilitated patients in hospital becomes colonized with Gram-negative bacilli that can be the source for Gram-negative pneumonia. Many Gram-negative infections can also be traced to respiratory therapy equipment, intravenous lines, catheters and other invasive equipment.[2]

Clinical Findings and Diagnosis

The clinical features of Gram-negative sepsis are similar to those of Gram-positive sepsis, and clinical signs cannot reliably distinguish between the two. A classic retrospective study of more than 600 patients with Gram-negative bacteremia at Boston University Hospital by Kreger, McCabe and co-workers provides important information on the frequency and prognostic significance of clinical findings.[5,6]

Although fever and chills are seen in most cases, 13% of patients are hypothermic at the onset of bacteremia, with

From the Division of Infectious Diseases, Department of Medicine, UCLA School of Medicine and Center for the Health Sciences, Los Angeles. Dr Jacobson is a Fellow in Infectious Disease. Dr Young is now at the Kuzell Research Institute of Pacific Medical Center, San Francisco.

Reprint requests to Mark A. Jacobson, MD, Room 37-121 CHS, UCLA Center for the Health Sciences, Los Angeles, CA 90024.

ABBREVIATIONS USED IN TEXT

ACTH = adrenocorticotropic hormone
CNS = central nervous system
CVP = central venous pressure
DIC = disseminated intravascular coagulation
LV dp/dt = first derivative of left ventricular pressure with respect to time

temperatures of less than 36.4°C (96.6°F), and 5% are afebrile.[6] Careful observation of critically ill patients has shown that hyperventilation is often the earliest clinical finding. A sudden onset of tachypnea is an indication for drawing blood for cultures in high-risk patients. Changes in mental state (either lethargy or agitation) are seen frequently.

A characteristic skin lesion, ecthyma gangrenosum, is seen in 1% to 25% of cases of *Pseudomonas* bacteremia and has also been observed with *E coli, Klebsiella* and other Gram-negative bacteremias.[7] Vesicular, bullous, erythematous and petechial lesions have also been described.[8] Findings obtained from Gram's stains and cultures of such lesions can indicate the initial microbiologic diagnosis.

Hypotension with oliguria, thrombocytopenia, leukopenia and abnormalities of coagulation are common findings, as shown in Table 1. In a series of hemodynamically monitored patients at Cook County Hospital (Chicago) with Gram-negative and Gram-positive bacteremia, the heart rate and cardiac index were significantly lower in those with Gram-negative bacteremia, but these findings are unlikely to be of diagnostic value.[9] At Boston University Hospital, 55% of patients with bacteremia had decreased platelets, but only 11% had laboratory evidence of disseminated intravascular coagulation (DIC), with only 3% experiencing clinically evident bleeding.[6] Neame and associates at McMaster University (Hamilton, Ontario), using sensitive tests for detecting thrombin and plasmin activation, found evidence of DIC in almost all patients with bacteremia who had platelet counts of less than 50,000 per μl.[10] Patients with thrombocytopenia in the 50,000 to 150,000 per μl range, however, had little evidence of DIC. Such moderate thrombocytopenia that commonly occurs in patients with Gram-negative bacteremia may be due to toxin-mediated endothelial cell damage or circulating immune complexes. Finally, major organ failure is not an infrequent direct result of Gram-negative sepsis.

TABLE 1.—*Signs and Symptoms of Gram-Negative Bacteremia**

Primary
Fever, 80%†
Chills
Hyperventilation (may be earliest finding)
Hypothermia, 13%†
Skin lesions
Mental state change
Complications
Hypotension, 44%†
Thrombocytopenia, 56%†
Clotting abnormalities, 31%†
Laboratory evidence of DIC, 11%†
Clinical DIC, 3%†
Leukopenia
Organ failure
Adult respiratory distress syndrome: cyanosis, acidosis
Acute tubular necrosis: anuria, acidosis
Liver: jaundice
Congestive heart failure

DIC = disseminated intravascular coagulation

*Modified from Young.[2]
†From Kreger et al.[6]

The diagnosis is proved by positive blood cultures. This presents a problem because Gram-negative bacteremia is relatively low grade and may be transient compared with endocarditis. Culture of blood specimens drawn after the appearance of fever may fail to detect transient bacteremia.

At Boston University Hospital, 77% of positive cultures had fewer than ten organisms per milliliter of blood.[5] When untreated bacteremic patients had cultures of three blood specimens drawn within 24 hours, 80% were positive in the first set and 99% had one of three positive.[11] In another study, the mean time interval to detect Gram-negative rods was three days. More than 90% of positive blood cultures had been recovered by the seventh day.[12] Hence, the "rule of threes." Three blood cultures and three days of observation will document most untreated cases of Gram-negative bacteremia. Observation for as long as two weeks and blind subculturing increases the yield.

Prognosis

The prognosis in Gram-negative bacteremia is mainly related to host, not pathogen, characteristics. McCabe and Jackson developed a classification of underlying host disease that has been used by many subsequent investigators to stratify patients into comparable groups.[12a] Such stratification makes the evaluation of therapeutic trials more meaningful. Nonfatal disease is defined as unlikely to be fatal within five years, ultimately fatal is likely to be fatal within five years and rapidly fatal applies to neutropenia and acute leukemia. At

TABLE 2.—*Factors Affecting the Outcome of Gram-Negative Bacteremia**

Underlying disease
Neutropenia
Hypogammaglobulinemia
Diabetes
Alcoholism/cirrhosis
Renal failure
Congestive heart failure
Respiratory failure
Antecedent therapy with steroids
Failure to mount febrile response
Complications of bacteremia at onset of treatment
Shock, anuria, DIC, abnormal coagulation
Antibiotic therapy
Antecedent antibiotics increase fatality
Appropriate antibiotic therapy decreases fatality by half
Severity of bacteremia
Polymicrobial
More than 10 organisms per ml
Serum-resistant organism
Source of infection
Interval to onset of therapy
Age over 60

DIC = disseminated intravascular coagulation

*Modified from Young.[2]

Boston University Hospital and in other studies, there has been a pronounced increase in fatality due to Gram-negative bacteremia with progressively more fatal underlying disease.[4-6,13] Other significant prognostic factors are listed in Table 2.

Normal human serum has bactericidal activity and most of a Gram-negative bacterial inoculum placed in serum will be killed by complement-mediated reactions. A serum-sensitive organism is defined as more than 90% killed by normal human serum versus a serum-resistant bacterium that is less than 90% killed. Most invasive Gram-negative bacteria are serum-resistant, and these are associated with an increased incidence of death and shock.[14] Other postulated microbial mechanisms of virulence include adherence to mucosal surfaces, antiphagocytic surface membranes and surface antigens similar to host antigens.

Pathophysiology

By far, the most significant virulence factor is endotoxin. The Gram-negative bacterial cell wall has three layers: an inner plasma membrane; a middle, rigid, mucopeptide solid membrane, and an outer lipoprotein lipopolysaccharide membrane. The exterior polysaccharide component of this outer membrane corresponds to the O antigen responsible for the serologic specificity of different Gram-negative bacteria. The interior core glycolipid consists of an oligosaccharide linked to a lipoidal glucosamine disaccharide common to all Gram-negative bacteria, termed lipid A. A number of animal and human experiments suggest that lipid A is the bacterial endotoxin responsible for generating the pathophysiologic complications such as shock, organ damage and DIC.[2]

The pathophysiology of Gram-negative bacteremia is still largely speculative. Applying the results of animal studies to human pathophysiology is limited by major species differences in mammalian response to endotoxin. For example, the Shwartzman reaction seen in rabbits is rarely seen in humans. Dogs experience a unique hypotensive phase following endotoxin administration that can be blocked by antihistamines. There is, however, very suggestive evidence that endotoxin produces septic shock and DIC by directly activating coagulation and complement cascades. Figure 1 outlines the complex humoral interactions set off by endotoxin. Although overly simplified, this figure gives some idea of the complex interactions involved. Activation of bradykinin and complement-mediated granulocyte degranulation probably causes the excess vasodilation and vascular permeability that result in hypotension. Histamine and endorphins may also play a significant role in causing hypotension. Granulocyte aggregation and intravascular coagulation may result in organ damage. Endotoxin also appears to have a direct depressing action on the myocardium and triggers the endothelial injury seen in Gram-negative bacteremia. Septic shock occurs when cardiac output is unable to maintain adequate blood pressure in a setting of loss of effective intravascular volume.

Treatment

Basic Adjunctive Therapy

The first step in adjunctive therapy for Gram-negative bacteremia is to maintain adequate tissue perfusion with volume replacement. In a hypotensive patient, central venous pressure (CVP) or Swan-Ganz monitoring is useful in determining when sympathetic amines are necessary and in avoiding fluid overload. When hypotension occurs, fluids should be given until CVP or wedge pressure is upper normal. If the patient is still hypotensive, blood pressure should be supported by administering sympathetic amines. Hemodynamic studies show that dopamine or dobutamine is preferable to norepinephrine or isoproterenol; the mean arterial pressure and cardiac index are maximized without intensive peripheral vasoconstriction.[9] Also, renal blood flow is maintained at doses of less than 12 μg per kg of body weight per minute. Dobutamine appears equally useful in this setting and may be preferable to dopamine when congestive heart failure or fluid overload supervenes because dopamine may increase wedge pressure and dobutamine has the opposite effect.[15] Of course, in patients with severe refractory hypotension, dopamine is superior to dobutamine in maintaining mean arterial blood pressure.

The treatment of DIC with heparin is controversial. Studies of both animal and human bacteremia show significant improvement in coagulopathy but no difference in mortality rates with heparin.[16,17] Bleeding due to DIC should probably be treated with replacement therapy: platelets for thrombocytopenia, cryoprecipitate for hypofibrinogenemia and fresh frozen plasma for decreased coagulation factors.

Drug Therapy

Antibiotics are considered paramount in the therapy for Gram-negative bacteremia, but the interpretation of clinical trials is difficult. The pooled results of three studies done before 1970 show significantly improved survival rates in patients with ultimately fatal or nonfatal disease when treated with an appropriate antibiotic. Patients with rapidly fatal dis-

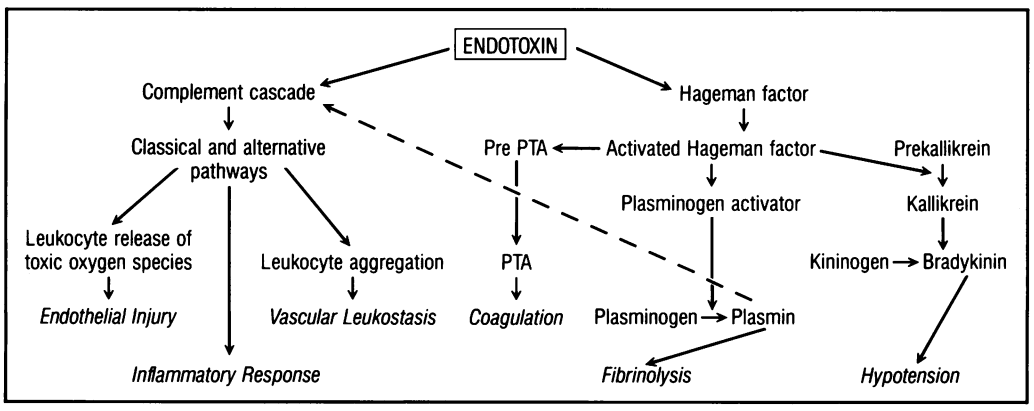

Figure 1.—Pathogenesis of septic shock (modified from Young[2]). PTA = plasma thromboplastin antecedent

ease had a 15% survival rate, irrespective of antibiotic therapy.[13] Love and colleagues at the Baltimore Cancer Research Program in the 1970s showed increased survival in patients with neutropenia whose pathogen was susceptible to the initial empiric antibiotics. The response was 44% in patients with susceptibility to one antibiotic and 75% in those whose pathogen was susceptible to two antibiotics.[18] On the other hand, the Boston University retrospective study showed no improvement in survival using a combination of antibiotics compared with the administration of a single drug. In 1971 an 84% response rate was reported in patients with bacteremic *Pseudomonas* infections treated with gentamicin and carbenicillin.[18-20] This greatly exceeded historical controls and ushered in the era of empiric multiple antibiotic therapy for febrile patients with neutropenia.

Combinations of aminoglycosides and β-lactams have been the mainstay of antimicrobial therapy for Gram-negative bacteremia in the past decade. There does not appear to be a single "best regimen." Response rates of up to 80% are obtained when amikacin is combined with ticarcillin, piperacillin, azlocillin or moxalactam.[19-21] There is no evidence that an aminoglycoside-penicillin-cephalosporin regimen is superior to a two-drug aminoglycoside-β-lactam combination.

The development of the third-generation cephalosporins and extended-spectrum penicillins raises the possibility of single or double β-lactam therapy for sepsis due to Gram-negative organisms. These new antibiotics have broad activity against Gram-negative rods and easily achieve bactericidal serum concentrations. They have the advantage of significantly less nephrotoxicity than aminoglycosides.

Monotherapy with third-generation cephalosporins appears to equal combined aminoglycoside-β-lactam regimens in treating Gram-negative bacteremia when patients with neutropenia and *Pseudomonas* infections are excluded.[22,23] This form of therapy is currently being examined in patients with neutropenia. Small studies in which moxalactam disodium, ceftazidime or cefoperazone sodium was given alone to infected neutropenic patients resulted in 60% to 80% response rates, which were comparable to the more standard regimens.[24,25] Potential problems with cephalosporin monotherapy include risk of rapid emergence of a resistant bacterial strain due to β-lactamase induction, poor enterococcal coverage and variable bactericidal effect against *P aeruginosa.* Monotherapy with extended-spectrum penicillins, such as mezlocillin, has been found to be inadequate.[26]

The use of two β-lactam antibiotics, or "double β-lactam therapy," has been examined in two studies. A combination of moxalactam and piperacillin equaled standard aminoglycoside-β-lactam therapy in response rate.[24,27,28] Synergism between two different β-lactam antibiotics may occur by the drugs binding different penicillin-binding proteins or by one drug inhibiting β-lactamase, thus allowing the other to achieve its full effect. Double β-lactam therapy offers the advantages of a wide spectrum of activity and a decreased incidence of resistant strains compared with monotherapy. Aminoglycoside toxicity is also avoided. Some combinations of cephalosporins and penicillins, however, may still induce β-lactamase production by Gram-negative rods such as *Pseudomonas, Enterobacter* and *Serratia.*[29]

The clinical response to antibiotic therapy in Gram-negative bacteremia may be predicted from antibiotic synergism and serum bactericidal activity. Anderson and co-workers at UCLA reviewed 173 cases of Gram-negative bacteremia treated with two antibiotics.[30] Antimicrobial synergism occurred when a fourfold or greater decrease in the concentration of each antibiotic used in combination inhibited growth of a test organism. The patients whose organisms were synergistically inhibited responded significantly better than those whose were not. This finding confirms clinical studies by Klastersky and colleagues that showed a positive correlation between the presence of synergism and favorable clinical response.[31] Peak serum bactericidal titers of 1:16 or more also correlate with favorable clinical response in patients with neutropenia and Gram-negative bacteremia.[32] Serum bactericidal activity may be another useful way to monitor combination therapy. Methods of determining synergism and bactericidal activity, however, have been difficult to standardize and results obtained too late to influence therapy.

There remain several other good arguments for using a combination of antibiotics in the empiric treatment of Gram-negative bacteremia in an impaired host. Combination therapy makes it possible to cover both Gram-positive and Gram-negative organisms (which cannot be reliably distinguished clinically). It may be three days or longer before cultures are positive. Polymicrobial bacteremia may be present. Combined antibiotics may also prevent the emergence of resistant organisms in the host.

To doubt the value of antibiotics in treating Gram-negative bacteremia would seem heresy. A recent review, however, of 1,186 episodes of Gram-negative bacteremia showed that *initial* appropriate antibiotics did not improve survival when compared with ineffective or no antibiotic therapy.[4] The efficacy of antibiotics given after the first calendar day on which blood cultures turned positive was clearly related to patient outcome, but initial antibiotic treatment was not. The authors concluded that this "apparent lack of effect of initial antimicrobial therapy would, then, amply justify attempts to devise novel methods of therapy."[4]

The remainder of our discussion concerns experimental adjunctive modes of therapy for Gram-negative bacteremia—modes that when given *early* in infection might arrest the pathophysiologic sequence of events leading to shock, organ failure and death.

Corticosteroids

The use of glucocorticoids in patients with septic shock has been a highly controversial subject for several decades. Some physicians believe that steroids can significantly improve the outcome of treatment for septic shock, especially when they are given early in the course of the infection and in pharmacologic doses, while others feel that the literature to date is inadequate in view of the potential risks of these drugs—that is, further impairing host defenses against extravascular foci of infection. In 1981 the Food and Drug Administration decided to remove septic shock as an indication for the use of high-dose methylprednisolone. Interest has been rekindled, however, by recent reports of the roles of endorphins and of complement-mediated granulocyte aggregation in sepsis.

Many studies of steroid therapy for bacteremia have major methodologic problems. In 1974 Weitzman and Berger reviewed the English language literature, examining 32 studies

in humans for adherence to eight standards of clinical trial design.[33] The eight standards they considered basic to proper experimental design and validity included (1) prospective rather than retrospective design, (2) the presence of concurrent, not historical, controls, (3) the use of random allocation, (4) the use of double-blind technique, (5) adhering to precise diagnostic criteria, (6) stratifying patients according to clinical extent of disease, (7) stratifying patients according to underlying disease and (8) the use of standard observations on the complications of therapy. Only 41% of the studies reviewed were prospective, 44% used concurrent controls, 25% were randomized and 16% were double-blind. Half or fewer of the studies met the remaining criteria. Most of the studies lacked consistency in details of steroid administration, such as dosage, duration of therapy or use of a single preparation. Of the 32 studies, 22 showed steroids to favorably influence the course of sepsis, but these generally had more design faults than those that detected no difference between treatment and control groups. The best study dealing with Gram-negative bacteremia in this group was by Klastersky and coworkers in Belgium in 1971.[34] It showed no difference in mortality rates between patients given placebo and those treated with betamethasone, 1 mg per kg a day for three days (equivalent to 7 mg per kg a day of methylprednisolone).

Since Weitzman and Berger's critique, there have been three major reports on the subject of steroid therapy for bacteremia. The first was published in the surgical literature by Schumer from the Chicago Veterans Administration Hospital.[35] This study met all eight methodologic standards, involved 172 consecutive patients with blood culture-proved septic shock, used higher dose steroids than did Klastersky and associates and administered therapy *at the time of diagnosis*. The mortality rates were significantly different: 10% in the steroid-treated group versus 38% in the placebo group. This study has been criticized for the following reasons: use of two steroid preparations (dexamethasone and methylprednisolone), failure to use a uniform antibiotic protocol, the use of chloramphenicol alone during the first phase and lack of data on adjunctive supportive measures.

There are substantial data from studies of animals suggesting that the early use of steroid therapy prevents the occurrence of the septic shock syndrome. But most of the animals used in shock experiments have not had the underlying diseases—that is, burns, leukemia, trauma—seen in the serious human clinical states that are frequently complicated by bacteremia. Human clinical trials have required septic shock to be fully developed before steroids are administered so that a variety of inflammatory responses have already been expressed by the time therapy is given. Treatment that inhibits these responses at a very early stage may be required to show a measurable effect on human mortality rates.

A recent prospective controlled, unblinded study by Sprung and colleagues suggests a significant effect of large corticosteroid doses in reversing septic shock.[36] Although mortality was unaffected, septic shock was reversed within 24 hours in 11 of 43 steroid-treated patients versus 0 of 16 controls ($P < .05$). Patients who received corticosteroids within four hours after onset of shock experienced a significantly higher incidence of shock reversal than those who were treated later.

Also of interest is a randomized, placebo-controlled, double-blind study conducted in Indonesia that showed a highly beneficial effect on mortality when patients with severe typhoid fever (manifested by shock, delirium or obtundation) were given high-dose dexamethasone (3 mg per kg followed by 1 mg per kg every six hours for two days).[37]

The empiric use of steroids may have a sound physiologic basis. As is discussed subsequently in this report, high doses of steroids appear to inhibit the release of β-endorphin as well as adrenocorticotropic hormone (ACTH) from the pituitary. This may counter endotoxin stimulation of endorphin release and blunt resultant hypotension. The inhibition of complement-mediated granulocyte aggregation has been recently proposed as a mechanism of action of high-dose steroids in septic shock.[38] Endotoxin activates the complement system, and it has been recently shown that granulocytes aggregate when exposed to activated complement, specifically C5a.[39] When cultured human umbilical vein endothelial cells were exposed to neutrophils activated by C5a, the neutrophils adhered to the endothelial cells and released toxic oxygen species, which resulted in endothelial cell damage. Cell damage was inhibited by hydrocortisone.

In one experiment, endothelial damage was measured by the cellular release of labeled chromium after cells were exposed to serum, endotoxins and leukocytes.[39] When hydrocortisone was added, this damage was significantly reduced. Endothelial damage and capillary leakage is the endpoint of several endotoxin-mediated pathways that result in hypotension in cases of Gram-negative bacteremia. If steroids interrupt this sequence of events, hypotension may be averted. In vitro and in vivo studies showed that corticosteroids do interrupt this sequence by blocking complement-mediated granulocyte aggregation. In one study, the approximate plasma concentration of methylprednisolone achieved by a dose of 30 mg per kg almost completely prevented in vitro complement-mediated aggregation.[40] Recent research at Johns Hopkins University (Baltimore) by Skubitz, Craddock and Hammerschmidt suggests that there is a specific complement receptor on the granulocyte surface.[41] Steroids slow the rate of complement-receptor association without affecting disassociation and thereby inhibit granulocyte aggregation in a dose-dependent manner. The following order of potency for this kinetic effect from the greatest to the least was methylprednisolone, hydrocortisone and dexamethasone. If steroids are to be used in treating for septic shock, this suggests that methylprednisolone is the drug of choice.

The ability of steroids to interrupt complement-granulocyte interaction is a function of the time of administration. When given before rather than after complement activation, steroids more effectively inhibit aggregation, and lower concentrations of the drugs are needed. In the future, rapid predictors of clinical deterioration, such as endorphin or C5a assays, may identify patients who are most likely to benefit from steroid therapy.

A recent study of the effect of high-dose corticosteroids on alveolocapillary permeability in human septic acute respiratory distress syndrome appears to confirm the importance of complement-mediated aggregation.[42] Steroids significantly decreased abnormal alveolocapillary permeability caused by bacteremia, presumably by interfering with complement-induced granulocyte aggregation. This effect was seen only in patients who were in the early stages of their illness. Methyl-

prednisolone acted more rapidly than dexamethasone in reducing permeability.

Future confirmatory double-blind prospective studies of high-dose methylprednisolone and controlled antibiotics may clarify the clinical value of steroids in septic shock. Patients most likely to benefit are those in shock who are treated early (within the first hour or two after hypotension has supervened) but who are without serious underlying or unremitting disease—analogous to laboratory animals or typhoid victims. Currently, we favor restricting administration of a single 30-mg-per-kg dose of methylprednisolone to patients suspected of having Gram-negative bacteremia who fit this profile. In this setting, the recent study of Sprung and co-workers suggested a significant superinfection rate attributable to dexamethasone. Methylprednisolone-treated patients, however, did not experience increased adverse effects compared with controls.[36]

Naloxone

Recent experimental evidence has directed attention to the role of endorphins in septic shock and the possible beneficial effect of naloxone in treating this disorder. Early research on endorphins focused on their role as opiate analogues that function as pain modulators. Naloxone, a pure opiate antagonist, was shown to block endorphin analgesia. Endorphins, like exogenous opiates, produce hypotension, and naloxone can also block this effect.

Endorphins are small peptide molecules secreted and stored in the pituitary gland. The specific molecules β-endorphin and ACTH are both derived from the same parent molecule and are stored in common cellular sites in the pituitary. Endotoxin appears to stimulate the release of both endorphin and ACTH from the pituitary, as do acute stress and adrenalectomy. As was discussed previously in this report in the section on Corticosteroids, the administration of corticosteroids inhibits ACTH and endorphin release.

A series of elegant animal studies carried out by Faden and Holaday at the Walter Reed Army Medical Center (Bethesda, Md) suggests that naloxone can reverse hypotension in patients with Gram-negative bacteremia and raises the possibility of a pathophysiologic mechanism.[43] Unanesthetized rats were given purified *E coli* endotoxin. When the mean pressure dropped to 65 mm of mercury, rats received either a placebo or naloxone. Naloxone restored the mean pressure to preendotoxin levels in minutes, but had no effect on mean pressure in rats that did not receive endotoxin. Prophylaxis with naloxone was less effective than when the drug was administered to an animal in shock, suggesting that endorphin modulation of blood pressure is not a tonically active system.

Naloxone maintained pressure when given as a continuous infusion, and a dose-response relationship was shown with maximal effect at 1 mg per kg. Experiments with levorotatory and dextrorotatory isomers of naloxone showed that the effect of naloxone on blood pressure after endotoxin administration occurred only with the levorotatory form. This stereospecificity suggests that the effect of naxolone is opiate receptor-mediated and not due to some other pharmacologic effect of the drug. Increased mean pressure was produced when a small amount of levonaloxone was injected into the cerebral ventricle. There was no effect when the same amount was given peripherally or when dextronaloxone was given intraventricularly. Thus, it appears that the effect of naloxone in shock is mediated by central nervous system (CNS) opiate receptors. Although naloxone increased the mean pressure in the rat-endotoxin model, it did not significantly change survival. This may indicate that its effect is species-specific, because similar studies in dogs showed a statistically significant increased survival over controls.[43,44]

The results of experiments using the canine endotoxic model suggest that the primary therapeutic effect of naloxone in endotoxic shock is to reverse depression of myocardial contractility.[43] The first derivative of left ventricular pressure with respect to time (LV dp/dt) was used as an index of left ventricular contractility. Mean arterial pressure, wedge pressure and cardiac output were also measured. Endotoxin produced a substantial decrease in all of these parameters. Naloxone-treated dogs differed from controls by increased LV dp/dt, cardiac output and mean arterial pressure. This improvement was not seen in peripheral resistance, venous return or heart rate, suggesting that the hypotensive effect of endorphins in shock is due to decreased myocardial contractility and that naloxone inhibits this effect. Because bilateral cervical vagotomy and intravenous administration of atropine both abolish the cardiovascular effects of intraventricular naloxone, the cardiodepressant effects of endorphins may result from a CNS-endorphin-parasympathetic pathway.

A study of rat cardiac papillary muscle described evidence of stereospecific opiate binding in heart muscle.[45] There may be both indirect CNS and direct cardiac endorphin effects on contractility. At McGill University, Montreal, another animal model was studied by Gahhos and associates.[46] Naloxone given to pigs two hours after the induction of *E coli* septic shock had only transient, relatively minor effects on blood pressure. The authors suggested that the hemodynamic effect of naloxone may occur only when given early in the course of bacteremia.

Data on the use of naloxone in human cases of septic shock have been anecdotal for the most part. There have been a number of reports in *The Lancet* of cases of severe septic hypotensive episodes unresponsive to pressors in which giving naloxone has resulted in dramatic recovery.[47-49] Doses used ranged from 0.01 to 0.1 mg per kg. There is one small pilot study from the Brigham and Women's Hospital (Boston) by Peters and colleagues[50] in which 13 patients with sustained hypotension, 10 of whom had sepsis as the cause, and either oliguria or impaired mental state were given naloxone intravenously. Therapy with pressors, antibiotics and fluids was continued unchanged. Of the 13, 4 had hypoadrenalism from either Addison's disease or long-term steroid administration. Eight septic patients who did not have hypoadrenalism had a 45% rise in systolic pressure within minutes of receiving 0.4 to 1.2 mg of naloxone. This increase lasted about 45 minutes (compared with a half-life for naloxone of about one hour). In two of these patients, a second dose resulted in another increase in blood pressure. The patients with hypoadrenalism had no pressor response to naloxone. Naloxone also had been described as effective in rat hypovolemia and spinal shock[43] and in one case report of human cardiogenic shock.[48]

The only potential adverse effect of naloxone in bacteremia is sympathoadrenal stimulation leading to excessive systemic vascular resistance.[44] In studies in animals this has not had a detrimental effect on mortality, and for some spe-

cies, such as the dog, it actually may enhance survival. There are rare case reports attributed to naloxone of ventricular arrhythmias and pulmonary edema in patients postoperatively but a causal relationship has not been established.[51]

In view of the safety of this drug and the literature just reviewed, a trial of naloxone therapy can be justified when septic shock is unresponsive to volume replacement and pressors. Our dosage approach is to give a 1-mg bolus and repeat until hypotension resolves or a maximum dose of 0.1 mg per kg is reached. When a clinical response occurs, a 1-mg-an-hour maintenance infusion is instituted. While naloxone appears to have a transient pressor effect in septic shock and causes minimal adverse effects, clinical trials are needed to establish the precise efficacy and the full range of dose-response in humans.

Granulocyte Transfusions

Gram-negative bacteremia is especially lethal in neutropenic patients. The incidence of Gram-negative bacteremia is inversely proportional to the total granulocyte count. The incidence increases with fewer than 1,000 granulocytes per μl and substantially so with counts of fewer than 500.

Although advances in antibiotic treatment in the past 20 years have improved the prognosis for patients with neutropenia who have Gram-negative bacteremia (see section on Antibiotic Therapy), the problem has been approached in the past decade from another direction, that of transfusing neutrophils to patients with neutropenia. There have been five prospective randomized controlled studies of therapeutic granulocyte transfusions for patients who have bacteremia and neutropenia (excluding neonates).[52-56] Three showed statistically improved survival in transfused patients, one showed no significant difference and one showed significantly improved survival only in the subset of patients with persistent marrow failure.

In 1975 Higby and co-workers at the University of Buffalo randomly assigned patients with clinically evident infection and fewer than 500 granulocytes to either control groups or to groups that would receive granulocyte transfusions.[52] Three-week survival between these two groups was significantly different: 5 of 19 controls compared with 15 of 17 for transfused patients. Patients were entered only if after 48 hours antibiotic therapy was deemed ineffective. There were two major weaknesses of this study: the specific antibiotics used were not documented and possibly not controlled for, and isolation of an organism was not required to document infection.

At Emory University (Atlanta) in 1977 Vogler and Winton randomly assigned 30 patients who had culture-proved infection, had fewer than 300 neutrophils, failed to respond to the administration of appropriate antibiotics for three days and had an available donor.[53] The median survival differed significantly in these patients; the control patients survived 8 days whereas those who were transfused survived 22 days. There was an even greater significant difference in survival in the subgroup of patients for whom marrow recovery did not occur during the study period. Of these patients with persistent neutropenia, 5 of 11 who were transfused responded to therapy versus 9 of 11 control subjects. Weaknesses of this study were that antibiotic usage was not rigorously controlled and there may have been a significant difference between control and experimental groups in the extent of underlying disease; six of the treated patients versus only two of controls recovered marrow function during the study period.

In 1977 Herzig and associates at the National Cancer Institute published the most convincing randomized study in favor of therapeutic granulocyte transfusions.[54] All of the 27 patients studied had fewer than 1,000 granulocytes and blood culture-proved Gram-negative bacteremia. All received the same doses of cephalothin, gentamicin and carbenicillin. In each case, organisms showed in vitro sensitivity to at least one of the antibiotics. Daily granulocyte transfusions were begun within 24 hours of a positive culture and continued until marrow recovered or infection was cured. During the study period, 5 of 14 controls survived compared with 12 of 16 transfused patients ($P < .04$). Long-term survival for patients who eventually recovered marrow function did not significantly differ between test and control groups; of the patients who remained neutropenic, however, 8 of the 12 who were transfused survived 1 to 18 months compared with none of the controls.

In 1982 Winston and colleagues at UCLA published the findings of a prospective randomized trial showing no significant survival advantage to granulocyte transfusions.[55] This study had twice the number of patients of any previous study. Among patients with documented Gram-negative bacteremia, 23 of 36 controls versus 19 of 32 transfused patients survived, which was not a significant difference. Almost 100% of patients who recovered marrow function survived in both groups, and about 50% of both control and transfused patients who continued to have neutropenia survived the study period. Criticisms of this study are threefold: the antibiotics administered, although appropriate and probably therapeutically equivalent, were not rigorously controlled; there was no mention of long-term survival, and there was a difference in the underlying disease. For example, there were significantly more patients with acute lymphocytic leukemia in the control group and more with acute myelogenous leukemia in the test group. The key issue, however, in comparing this study with previous ones is the high survival of controls with persistent neutropenia: 50% in this study compared with 0% in the National Cancer Institute and Emory University studies. In a historically controlled, retrospective study, 37% of patients with persistent neutropenia who received antibiotics alone responded to therapy.[18]

Because the number of granulocytes per transfusion was similar in all studies, this comparison suggests that antibiotic therapy may have been suboptimal in earlier studies. The UCLA study differed from previous ones in that amphotericin B was used for patients who remained febrile after receiving combination antibiotics for seven days. Also, aminoglycoside levels may have been more carefully monitored.

Neonates with bacteremia, neutropenia and transient marrow neutrophil depletion appear to be a unique patient subset. Several studies suggest a pronounced survival benefit with minimal side effects when granulocyte transfusions are given to such infants.[57,58]

Adverse Effects

Granulocyte transfusions are not completely benign. Adverse effects that have been described include fever and chills

(which are common) and reversible acute respiratory distress, presumably due to sequestration of agglutinated neutrophils in pulmonary capillaries. There have been reports of cases of cytomegalovirus, toxoplasmosis and malaria being transferred by transfusion and of fatal graft-versus-host disease in patients who received nonirradiated cells.[59] A retrospective report from the National Cancer Institute documented a significantly higher incidence of acute respiratory deterioration when granulocyte transfusions were combined with amphotericin B, especially when the drug was added to the regimen of those patients who were already receiving transfusions. This reaction contributed to death in five cases and diffuse intra-alveolar hemorrhage was seen at autopsy.[60]

Another negative aspect of granulocyte transfusions is the high cost of the procedure. One analysis estimated the cost effectiveness of therapeutic transfusions in preventing death in patients with acute leukemia at $15,000 per life-year.[61] Therapeutic transfusions add 11% to the hospital bill of the average patient with leukemia. In light of these figures, it would be advantageous to identify a subgroup of patients with neutropenia who would more clearly benefit from transfusions.

Opsonin Activity

An interesting series of experiments from Mount Sinai Hospital (New York) by Keusch and co-workers suggests that serum opsonic activity may predict the outcome of treatment with granulocyte transfusions in infected patients with neutropenia.[62] Neutrophils require the presence of serum opsonins to effectively phagocytize bacteria. Early in infection, opsonization is primarily a function of the complement component C3b. Later on, immunoglobulins play an important role. These investigators measured serum opsonin levels in infected patients with granulocytopenia before the initiation of granulocyte transfusions. When human granulocytes were incubated with *E coli* in the absence of serum, there were no intracellular phagocytized bacteria seen by electron microscopy. When granulocytes were incubated with *E coli* and an 8% solution of normal human serum, bacteria were present within neutrophil vesicles, showing the opsonic activity of normal human serum. In this study, opsonins were measured by the serum-dependent uptake of radiolabeled bacteria by normal neutrophils. Eight of ten patients with 75% or more of standard serum opsonic activity against their own blood stream isolates responded to transfusions; none with less than 75% of standard activity responded.

Criteria for Patient Selection

In summary, optimal antibiotic therapy with careful monitoring of serum levels and organism sensitivities appears to eliminate the need for granulocyte transfusions in most cases. There are, however, several small prospective randomized studies that suggest a survival advantage for transfused infected neutropenic adults. In the future, opsonin assays may help to identify subgroups more likely to respond, and opsonin-deficient patients may be helped by antibody supplementation.

Currently, granulocyte transfusion therapy should not be considered for adults, unless the following minimal criteria are met[63]:

- Absolute neutropenia, with fewer than 500 total neutrophils.
- Culture-proved bacterial infection unresponsive to appropriate antibiotic therapy for at least 48 hours.
- Marrow recovery not expected for at least seven days (studies show best survival advantage in patients with prolonged neutropenia).

Immunization and Antiserum

An exciting new approach to therapy is the enhancement of host antibody response to Gram-negative bacilli. Serum complement and immunoglobulins, for example, are necessary for phagocytosis of bacteria, and serum opsonin levels may predict the outcome in some cases of Gram-negative bacteremia, as just described in the section on Granulocyte Transfusions.

At Memorial-Sloan Kettering (New York) in the early 1970s, an attempt was made to enhance serum opsonizing activity in cancer patients using a *Pseudomonas* vaccine derived from the lipopolysaccharide of seven *P aeruginosa* serotypes.[64] In a prospective randomized trial, the mortality rate associated with *Pseudomonas* infection was significantly lower in the treated group. There was a high incidence of adverse reactions, however, and immunity was short-lived. In India where topical antibiotics were unavailable, use of a *Pseudomonas* vaccine in burn patients greatly decreased mortality.[65]

Current interest has focused on enhancing the protective effect of host antibody to bacterial endotoxin. This interest was stimulated in part by the report of McCabe and coworkers in 1972, which related different host antibody titers to the frequency of shock and fatal outcome in 175 patients with Gram-negative bacteremia.[66] At the time bacteremia was first diagnosed or suspected, serum antibody titers were measured to three Gram-negative cell-wall antigens: O-specific antigen and two shared cross-reactive antigens, CA and Re. O antigen is determined by the polysaccharide component of the outer membrane and is unique to each bacterial strain. Re antigen is a shared cross-reactive antigen associated with core glycolipid. Mutant Re bacteria lack both the polysaccharide O antigen and the outer core structures and thus have core glycolipid exposed. Hence, antibody to Re antigen is essentially antibody to the core glycolipid common to all Gram-negative bacilli. Because core glycolipid is presumed to be Gram-negative endotoxin, antibody to Re antigen is presumed to be an antiendotoxin antibody.

In the study by McCabe and associates, the height of O and CA antibody titers did not correlate with clinical outcome, but death and shock were less frequent in patients with high titers of Re antibody. The implication of this study was that antibody to endotoxin was protective in Gram-negative bacteremia and that perhaps resistance to infection could be enhanced by immunization against endotoxin.

The most important work in this area has been done at the University of California at San Diego by Braude and colleagues with a mutant form of *E coli* designated J5.[67] J5 bacteria lack an enzyme necessary to incorporate galactose into cell wall lipopolysaccharide, which prevents the attachment of side chains to core glycolipid. The exposed core is presumed to be antigenic. In healthy rabbits vaccinated with killed J5 *E coli,* high levels of antibody to J5 developed. Antiserum harvested from these vaccinated rabbits protected other rabbits from the toxic effects of injected endotoxin.

Giving antiserum to neutropenic rabbits with bacteremia greatly improved survival compared with giving controls nonimmune serum.

This group later reported results of a multicenter, double-blind, randomized, prospective trial of J5 antiserum in patients with Gram-negative bacteremia.[68] Human J5 antiserum was obtained from healthy young men vaccinated with boiled J5 *E coli* cells. Control serum was also obtained from each volunteer before vaccination. Patients were enrolled if they appeared septic and had a high probability of Gram-negative infection. Each participant received one unit of control serum or antiserum at the time of enrollment. Among patients with documented Gram-negative infections, the mortality rate was 22% for those who received antiserum versus 39% for controls ($P = .01$). In the subgroup with profound shock, the mortality rate was 44% versus 77% for controls ($P = .03$).

These data are impressive, and antiserum may soon play an important role in the therapy for Gram-negative bacteremia. The fact that antiserum was most effective in patients who did not have neutropenia and in those with profound hypotension supports the theory that antiserum acts by binding to core glycolipid and blocking its access to host mediators of shock. J5 antiserum and active J5 immunization have also been protective against *Hemophilus influenzae* type b infections in mouse studies.[69]

A major problem is availability of antiendotoxin antibodies. Some commercial preparations of γ-globulin modified for intravenous use contain augmented levels of such antibodies, but human efficacy data are not yet available.[70]

An alternative to immunization and plasmapheresis of human donors is in vitro production of antibodies. Recently, human splenic lymphocytes were fused to a mutant human myeloma cell line to produce a hybridoma that secretes IgG antibody reactive with *H influenzae* capsular polysaccharide. Monoclonal antibodies from this clone are protective in an animal model of *H influenzae* infection.[71] Monoclonal antibodies to Gram-negative lipopolysaccharide have also been developed and appear protective in animal models.[72,73] Human J5 antiserum, human monoclonal antiendotoxin antibodies or antigen-binding fragments derived from animals may become an important part of the therapy undertaken for Gram-negative bacteremia in the near future.[74]

REFERENCES

1. Waisbren BA: Bacteremia due to gram-negative bacilli other than the Salmonella. Arch Intern Med 1951; 88:467-488

2. Young LS: Gram-negative sepsis, chap 54, *In* Mandell GL, Douglas GR, Bennett JE (Eds): Principles and Practice of Infectious Disease. New York, John Wiley, 1985, pp 452-475

3. Wolff SM: The treatment of gram-negative bacteremia and shock. N Engl J Med 1982; 307:1267-1268

4. Bryan CS, Reynolds KL, Brenner ER: Analysis of 1186 episodes of gram-negative bacteremia in non-university hospitals: The effects of antimicrobial therapy. Rev Infect Dis 1983; 5:629-638

5. Kreger BE, Craven DE, Carling PC, et al: Gram-negative bacteremia—III. Reassessment of etiology, epidemiology, and ecology in 612 patients. Am J Med 1980; 68:332-343

6. Kreger BE, Craven DE, McCabe WR: Gram-negative bacteremia—IV. Re-evaluation of clinical features and treatment in 612 patients. Am J Med 1980:344-355

7. Dorff GJ, Geimer NE, Rosenthal DR, et al: *Pseudomonas* septicemia, illustrated evolution of its skin lesion. Arch Intern Med 1971; 128:591-595

8. Fisher KW, Berger B, Keusch GT: Subepidermal bullae secondary to *Escherichia coli* septicemia. Arch Dermatol 1974; 110:105-106

9. Winslow EJ, Loeb HS, Rahimtoola SH, et al: Hemodynamic studies and results of therapy in 50 patients with bacteremic shock. Am J Med 1973; 54:421-432

10. Neame PB, Kelton JG, Walker IR, et al: Thrombocytopenia in septicemia: The role of disseminated intravascular coagulation. Blood 1980; 56:88-92

11. Washington JA II: Blood cultures: Principles and techniques. Mayo Clin Proc 1975; 50:91-98

12. Young LS, Martin WJ, Meyer RD, et al: Gram-negative bacteremia: Microbiologic, immunologic and therapeutic considerations. Ann Intern Med 1977; 86:456-471

12a. McCabe WR, Jackson GC: Gram-negative bacteremia. Arch Intern Med 1962; 110:856-866

13. Bryant RE, Hood AF, Hood CE, et al: Factors affecting mortality of gram-negative rod bacteremia. Arch Intern Med 1971; 127:120-128

14. McCabe WR, Kaijser B, Olling S, et al: *Escherichia coli* in bacteremia: K and O antigens and serum sensitivity of strains from adults and neonates. J Infect Dis 1978; 138:33-41

15. Jardin F, Sportiche M, Bazin M, et al: Dobutamine: A hemodynamic evaluation in human septic shock. Crit Care Med 1981; 9:329-332

16. Corrigan JJ, Kiernal JF: Effect of heparin in experimental gram-negative septicemia. J Infect Dis 1975; 131:138-143

17. Corrigan JJ: Heparin therapy in bacterial septicemia. Pediatrics 1977; 91:695-700

18. Love LJ, Schimpf SC, Schiffer CA, et al: Improved prognosis for granulocytopenic patients with gram-negative bacteremia. Am J Med 1980; 68:643-648

19. DeJongh CA, Wade JC, Schimpf SC, et al: Empiric antibiotic therapy of suspected infection in granulocytopenic cancer patients. Am J Med 1982; 73:89-96

20. Wade JC, Schimpf SC, Newman KA, et al: Piperacillin or ticarcillin plus amikacin: A double-blind prospective comparison of empiric antibiotic therapy for febrile granulocytopenic cancer patients. Am J Med 1981; 71:983-990

21. Gaya H: Rational basis for the choice of regimens for empirical therapy of sepsis in granulocytopenic patients. Schweiz Med Wochenschr 1983; 14(Suppl):49-57

22. Warren JW, Miller EH, Fitzpatrick B, et al: A randomized, controlled trial of cefoperazone vs. cefamandole-tobramycin in the treatment of putative, severe infections with gram-negative bacilli. Rev Infect Dis 1983; 5S:173-180

23. Klastersky J: The role of the new penicillins and cephalosporins in the management of infection in granulocytopenic patients. Schweiz Med Wochenschr 1983; 14(Suppl):64-69

24. Pizzo PA, Commers J, Cotton D, et al: Approaching the controversies in antibacterial management of cancer patients. Am J Med 1984; 76:436-449

25. Boliver R, Fainstein V, Elting L, et al: Cefoperazone for the treatment of infections in patients with cancer. Rev Infect Dis 1983; 5(Suppl):181-187

26. Wade JC, Schimpf SC, Newman KA, et al: Potential of mezlocillin as empiric single-agent therapy in febrile granulocytopenic cancer patients. Antimicrob Agents Chemother 1980; 18:299-306

27. DeJongh C, Joshi J, Newman K, et al: Moxalactam Plus Piperacillin or Amikacin: Empiric Therapy for Febrile Neutropenic Cancer Patients, abstract No. 943. Proceedings and abstracts of the 22nd Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, Miami Beach, 1982

28. Winston DJ, Barnes RC, Ho WG, et al: Moxalactam plus piperacillin versus moxalactam plus amikacin in febrile granulocytopenic patients. Am J Med 1984; 77:442-449

29. Sanders CC, Sanders WE: Microbial resistance to newer generation β-lactam antibiotics: Clinical and laboratory implications. J Infect Dis 1985; 151:399-406

30. Anderson ET, Young LS, Hewitt WL, et al: Antimicrobial synergism in the therapy of gram-negative rod bacteremia. Chemotherapy 1978; 24:45-54

31. Klastersky J: Empiric treatment of infections in neutropenic patients with cancer. Rev Infect Dis 1983; 5(Suppl):21-31

32. Sculier JP, Klastersky J: Significance of serum bactericidal activity in gram-negative bacillary bacteremia in patients with and without granulocytopenia. Am J Med 1984; 76:429-435

33. Weitzman S, Berger S: Clinical trial design in studies of corticosteroids for bacterial infections. Ann Intern Med 1974; 81:36-42

34. Klastersky J, Cappel R, Debusscher L: Effectiveness of betamethasone in management of severe infections. N Engl J Med 1971; 284:1248-1250

35. Schumer W: Steroids in the treatment of clinical septic shock. Ann Surg 1976; 184:333-341

36. Sprung CL, Caralis PV, Marcial EH, et al: The effects of high-dose corticosteroids in patients with septic shock: A prospective controlled study. N Engl J Med 1984; 311:1137-1143

37. Hoffman SL, Punjabi NH, Kumala S, et al: Reduction of mortality in chloramphenicol-treated severe typhoid fever by high-dose dexamethasone. N Engl J Med 1984; 310:82-88

38. Sheagren JN: Septic shock and corticosteroids (Editorial). N Engl J Med 1981; 305:456-457

39. Jacob HS, Craddock PR, Hammerschmidt DE, et al: Complement-induced granulocyte aggregation. N Engl J Med 1980; 302:789-794

40. Hammerschmidt DE, White JG, Craddock PR, et al: Corticosteroids inhibit complement-induced granulocyte aggregation. J Clin Invest 1979; 63:798-802

41. Skubitz KM, Craddock PR, Hammerschmidt DE, et al: Corticosteroids block binding of chemotactic peptide to its receptor on granulocytes and cause disaggregation of granulocyte aggregates in vitro. J Clin Invest 1981; 68:13-20

42. Sibbald WJ, Anderson RR, Reid B, et al: Alveolo-capillary permeability in human septic ARDS—Effect of high-dose corticosteroid therapy. Chest 1981; 79:133-142

43. Faden AI, Holaday JW: Experimental endotoxin shock: The pathophysiologic function of endorphins and treatment with opiate antagonists. J Infect Dis 1980; 142:229-238

44. Hinshaw LB, Beller BK, Chang AC, et al: Evaluation of naloxone for therapy of *Escherichia coli* shock—Species differences. Arch Surg 1984; 119:1410-1418

45. Burnie J: Naloxone in shock (Letter). Lancet 1981; 1:942

46. Gahhos FN, Chiu RC, Hinchey EJ, et al: Endorphins in septic shock. Arch Surg 1982; 117:1053-1057

47. Swinburn WR, Phelan P: Response to naloxone in septic shock (Letter). Lancet 1982; 1:167

48. Dirksen R, Otten MH, Wright DJ, et al: Naloxone in shock (Letter). Lancet 1980; 2:1360

49. Tiengo M: Naloxone in irreversible shock (Letter). Lancet 1980; 2:690

50. Peters WP, Johnson MW, Friedman PA, et al: Pressor effect of naloxone in septic shock. Lancet 1981; 1:529-532

51. Physicians' Desk Reference, 38th Ed. Oradell, NJ, Medical Economics, 1984, pp 925-926

52. Higby DJ, Yates JW, Henderson ES, et al: Filtration leukopheresis for granulocyte transfusion therapy. N Engl J Med 1975; 292:761-766

53. Vogler WR, Winton EF: A controlled study of the efficacy of granulocyte transfusions in patients with neutropenia. Am J Med 1977; 63:548-555

54. Herzig RH, Herzig GP, Graw RG Jr, et al: Successful granulocyte transfusion therapy for gram-negative septicemia. N Engl J Med 1977; 296:701-705

55. Winston DJ, Ho WG, Gale RP: Therapeutic granulocyte transfusions for documented infections. Ann Intern Med 1982; 97:509-515

56. Alavi JB, Root RK, Djerassi I, et al: A randomized clinical trial of granulocyte transfusions for infection in acute leukemia. N Engl J Med 1977; 296:706-711

57. Christensen RD, Rothstein G, Anstall HB, et al: Granulocyte transfusions in neonates with bacterial infection, neutropenia and depletion of mature marrow neutrophils. Pediatrics 1982; 70:1-6

58. Laurenti F, Ferro R, Isacchi G, et al: Polymorphonuclear leukocyte transfusion for the treatment of sepsis in the newborn infant. J Pediatr 1981; 98:118-123

59. Wintrobe MM, Lee GR, Boggs DR, et al: Clinical Hematology. Philadelphia, Lea & Febiger, 1981, p 512

60. Wright DE, Robichaud KJ, Pizzo PA, et al: Lethal pulmonary reactions associated with the combined use of amphotericin B and leukocyte transfusions. N Engl J Med 1981; 304:1185-1189

61. Rosenshein MS, Farewell VT, Price TH, et al: The cost-effectiveness of therapeutic and prophylactic leukocyte transfusion. N Engl J Med 1980; 302:1058-1062

62. Keusch GT, Ambinder EP, Kovacs I, et al: Role of opsonins in clinical response to granulocyte transfusion in granulocytopenic patients. Am J Med 1982; 73:552-563

63. Dinubile MJ: Granulocyte transfusion (Letter). Ann Intern Med 1983; 97:114

64. Young LS, Meyer RD, Armstrong D: *Pseudomonas aeruginosa* vaccine in cancer patients. Ann Intern Med 1973; 79:518-527

65. Jones RJ, Roe EA, Gupta JL: Low mortality in burned patients in a *Pseudomonas* vaccine trial. Lancet 1978; 2:401-403

66. McCabe WR, Kreger BE, Johns M: Type-specific and crossreactive antibodies in gram-negative bacteremia. N Engl J Med 1972; 287:261-267

67. Braude AI, Ziegler EJ, McCutchan JA, et al: Immunization against nosocomial infection. Am J Med 1981; 70:463-466

68. Ziegler EJ, McCutchan JA, Fierer J, et al: Treatment of gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia coli*. N Engl J Med 1982; 307:1225-1230

69. Marks MI, Ziegler EJ, Douglas H, et al: Induction of immunity against lethal *Haemophilus influenza* type b infection by *Escherichia coli* core lipopolysaccharide. J Clin Invest 1982; 69:742-749

70. Roby RE, Collins MS: Therapy of *Pseudomonas aeroginosa* Infection With *Pseudomonas* Hyperimmune IgG and Antibiotics in Leukopenic Mice, abstract No. 94. Proceedings and abstracts of the 24th Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, Washington, DC, 1984

71. Hunter KW Jr, Fischer GW, Hemming VG, et al: Antibacterial activity of a human monoclonal antibody to *Haemophilus influenzae* type b capsular polysaccharide. Lancet 1982; 2:798-799

72. Kim KS, Green D, Cross A, et al: Studies of the Protective Mechanism of Monoclonal Antibodies Against *E coli*, abstract No. 92. Proceedings and abstracts of the 24th Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, Washington, DC, 1984

73. Young LS: Immunoprophylaxis and serotherapy of bacterial infections. Am J Med 1984; 76:664-671

74. Antiserum for gram-negative bacteraemia (Editorial). Lancet 1983; 1:967-968